

# Modification of Carbon Paste Electrode with Molecularly Imprinted Poly(Gluthamic Acid) for Determination of Rhodamine: A Preliminary Study

Henry Setiyanto<sup>1</sup>, Ferizal<sup>2</sup>, Muhammad Yudhistira Azis<sup>3</sup>, Muhammad Ali Zulfikar<sup>4</sup>,  
Vienna Saraswati<sup>5</sup>, Nandang Mufti<sup>6</sup>  
{henry@chem.itb.ac.id<sup>1</sup>}

Analytical Chemistry Research Group, Institut Teknologi Bandung<sup>1,2,3,4</sup>

**Abstract.** The modification of carbon paste electrode (CPE) with molecularly imprinted polymer (MIP) for the determination of rhodamine-B by potentiometry method has been studied. The experiments were performed using glutamic acid as a monomer. The composition of monomers, the number of electropolymerization cycles and pH of the solution were used to describe modification electrodes made (CPE-MIP's) and the electrode characteristics were evaluated. It was observed the amount of oxidation current signal increase with increasing glutamic acid concentration. The optimal polymerization molar ratio of the functional monomer to the template molecule rhodamine-B was 3:1; the maximum number of electropolymerization was 15 and the optimum pH of supporting electrolyte solution was 4.

**Keywords:** CPE, MIP, Polyglutamic Acid, Rhodamine-B

## 1 Introduction

Rhodamin B is a synthetic dye that has high solubility in water and has a bright color. Rhodamin B is often used for food dyes such as fruit juices and preserved fruit [1], crackers, syrups, sweets, various kinds of cakes and is often used for cosmetic dye such as lipstick [2] and ink [3]. The use of rhodamin B as a food dye in some countries has been banned because its effects can interfere with human health including skin, eyes and respiratory irritations. Further accumulation can cause cancer, liver, kidney, and lymph damage [4]. In Indonesia, rhodamine B has been banned for use in food additives according to the Regulation of the Minister of Health No. 239 / Men.Kes / Per / V / 85. Until now, it is still often reported the use of rhodamine B for food dyes, especially for cheap market snacks.

Determination of rhodamine B can be done by UV-Vis spectrophotometry method [5], high-performance liquid chromatography [6], solid-phase extraction [2], high-performance liquid chromatography / UV-Vis [7], high-performance liquid chromatography/HPLC-FLD [8], liquid chromatography-mass spectroscopy [9]. Methods for determining rhodamine B have the disadvantages of having to prepare difficult test samples, expensive reagents, time-consuming and less selective. One method that can be used in determining rhodamine B is the electrochemical method. This method has succeeded in developing techniques for analyzing organic compounds [10],[11] as well as their degradation [12],[13]. This technique has the

advantage of being easy in the preparation process, cheaper, small in size, and allows it to have a very small detection limit, and also easy to automated.

The potentiometric method is one part of the electrometry technique. This method uses the principle of measuring electrical potential using several electrodes [14],[15],[16],[17]. The working electrodes used in this technique can use carbon paste electrodes (CPE) which can be modified with a polymer film on its surface to increase its sensitivity and selectivity. Polymer films or membranes can be modified by molding from target molecules which are often called molecularly imprinted polymers (MIPs). Some researchers have succeeded in developing MIPs and other membranes for the purposes of separation [18],[19],[20].

## **2 Experimental**

### **2.1 Reagents, Solution, and Samples**

The chemicals used in this study were rhodamine B, paraffin oil, graphite powder, L-glutamic acid, potassium hexacyanoferrate  $K_3[Fe(CN)_6]$ (III), potassium hexacyanoferrate  $K_4[Fe(CN)_6]$ (II), sodium hydrogen phosphate/ $Na_2HPO_4$ , sodium dihydrogen phosphate/ $NaH_2PO_4 \cdot H_2O$ , sodium hydroxide/ $NaOH$ , Na-benzoate, monosodium glutamate,  $H_3PO_4$  and distilled water. These reagents are available in the chemistry department laboratory. A stock solution of 0.01 M  $K_3[Fe(CN)_6]$ , 0.01 M  $K_4[Fe(CN)_6]$ , and 0.1 M  $NaCl$  as an electrolyte solution were prepared in distilled water. Phosphate buffers were used as supporting electrolytes for removing rhodamine B from the electrode surface. Sodium hydroxide was used as the supporting electrolyte to dissolve rhodamine B.

### **2.2 Preparation of Reference Electrode**

In the making reference electrode (Ag/AgCl electrode), the first silver wire is sanded then electrolyzed with 0.1 M  $NaCl$  solution at a fixed potential of 2 V for 10 minutes until a black AgCl layer will be formed on the surface of the Ag wire. In this process, Ag wire functions as a working electrode and platinum (Pt) wire functions as a reference electrode. Furthermore, the electrolyzed Ag wire is inserted into the electrode body which has been filled with 3 M  $NaCl$  solution and characterized in a 0.01 M  $K_3[Fe(CN)_6]$  and  $K_4[Fe(CN)_6]$  solution in 0,1 M  $NaCl$ . Characterization was performed using the cyclic voltammetry (CV) method in the potential range of -200 mV to 800 mV with a scan rate of 100 mV/s for 1 cycle. The characterization results compared with BAS commercial reference electrode.

### **2.3 Preparation of Carbon Paste Electrode (CPE)**

Graphite powder and liquid paraffin in the ratio of 7: 3 were placed on a watch glass, then heated to a temperature of 80 °C. After the temperature reaches 80 °C, graphite and paraffin were then mixed. The formed paste was then inserted into the electrode body containing copper wire. The surface of the carbon paste was smoothed by rubbing it on clean paper.

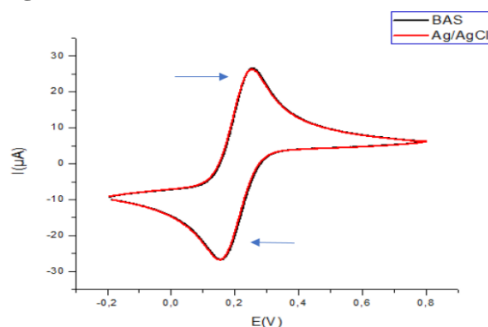
## 2.4 Preparation of Carbon Paste Electrode Modified MIPs (CPE-MIPs)

MIPs solution was prepared by mixing 7.5 mL of 0.01 mM glutamic acid solution, and 2.5 mL of 0.01 M rhodamine B solution in a 25 ml measuring flask and then diluted using a 0.01 M phosphate buffer pH 7. NIPs solution is made in the same way as MIPs without the use of rhodamine B. The electropolymerization process is carried out in MIPs/NIPs solutions. The process was carried out using cyclic voltammetry techniques with a range of -0.2 V – 1.8 V for 15 cycles in a 0.1 M phosphate buffer pH 7. The process of removing rhodamine B from the polymer matrix to obtain analytes molecular molds was carried out by means of a MIPs modified carbon paste electrode electrolyzed in a 0.1 M phosphate buffer pH 7 using a cyclic voltammetry technique at a potential of -0.2 V – 1.8 V of 15 cycles with a scan rate of 100 mV/sec.

## 3 Results and Discussion

### 3.1 Characterization of Reference Electrodes

In this study, rhodamine B measurements were carried out using 3 electrodes that are Pt wire as an auxiliary electrode, CPE-MIPs as a working electrode, and Ag/AgCl electrode as a reference electrode. Ag/AgCl electrode is used because this electrode has good stability and easy in the manufacturing process. Ag/AgCl reference electrode was characterized using  $K_3Fe(CN)_6$  and  $K_4Fe(CN)_6$  solution with ratio of 1:1 in 0.1 M NaCl solution by cyclic voltammetry method in the potential range of -0,2 V to 0,8 V for 1 cycle. Furthermore, the results of the Ag/AgCl electrode's characterization compared with the BAS electrodes. The results of the characterization of these electrodes must resemble (coincide) in order to show the same quality. This is important to ensure there are no errors in the measurements that can be caused due to the poor quality of the comparison electrodes. The cyclic voltammogram of these electrodes is presented in **Figure 1**.



**Fig. 1.** Cyclic voltammogram of Ag/AgCl electrode and BAS electrode in solution mixture of  $K_3Fe(CN)_6$  and  $K_4Fe(CN)_6$  in 0.1 M NaCl solution.

Based on the voltammogram in **Figure 1**, it can be seen that the Ag/AgCl electrode and BAS electrode are very coincided. Anodic and cathodic peak current values resulting from Ag/AgCl electrode characterization are shown in Table 1.

**Table 1.** The values of peak characteristic of Ag/AgCl and BAS electrodes.

Electrode	I <sub>pa</sub> (μA)	I <sub>pc</sub> (μA)	E <sub>pa</sub> (V)	E <sub>pc</sub> (V)	E <sup>0</sup> (V)
BAS	27.73	23.64	0.270	0.124	0.146
Ag/AgCl	27.88	24.22	0.266	0.114	0.152

### 3.2 Determination of the optimum composition of the monomer-analyte

To produce modifications with optimum results, one of the parameters studied is the ratio of monomer: analyte. The composition of the monomer and analyte in molecularly imprinted polymers/MIPs will determine the number of analytic molds formed in the polymer. In this study, the concentration of the analyte in the MIPs solution was made equal to 1 mM while the monomer concentration varied from 1 mM, 2 mM, and 3 mM.

Shown on Table 2, EPK-MIPs which give the highest peak current is EPK-MIP with 1 mM rhodamine B and 3 mM glutamic acid. The greater concentration of glutamic acid in the MIPs solution, the greater the surface area of the polymer so that the rhodamine B mold will be more numerous. However, if the polymer formed is too thick, it will be difficult for the process of removing rhodamine B or mold formation in EPK-MIPs so that the peak current produced will be lower.

**Table 2.** The composition of monomer-analyte (glutamic acid-rhodamine B).

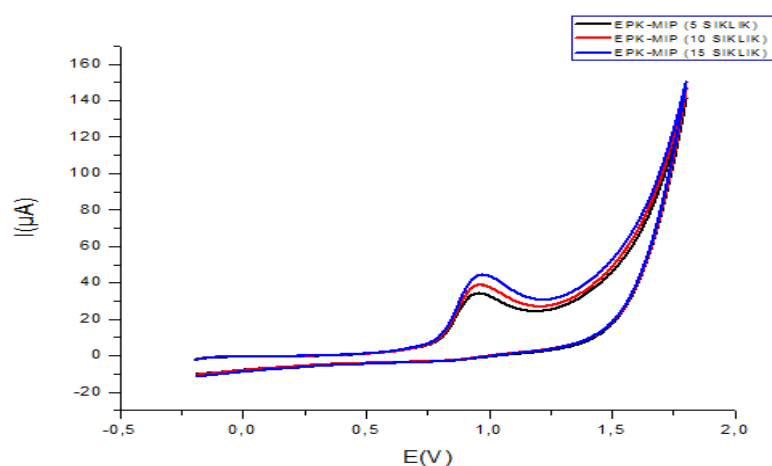
Monomer: analyte	I <sub>p</sub> (μA)	E(V)
1:1	34.38	0.9600
2:1	35.45	0.9600
3:1	40.57	0.9600

### 3.3 Determination of The Optimum Electropolymerization Cycle

The next parameter that affects the performance of the electrode modification is the number of electropolymerization cycles. The parameter cycles determine the thickness of the polymer layers formed. The thickness of the polymer layer on the surface of the electrode will affect the electrocatalytic properties of carbon paste electrode to rhodamin B. Thickness that is too thin or too thick will affect the peak current of rhodamine B so that the measurement of this compound becomes less sensitive. In this study variations in the number of electropolymerization cycles were done in 5 cycles, 10 cycles and 15 cycles. The effect of the thickness of the poly (glutamic acid) layer on the oxidation peak current of 1 mM rhodamine B in a 0.1 M phosphate buffer pH 7 on measurements using carbon paste electrodes modified with MIPs are shown in Table 3 and **Figure 2**.

**Table 3.** Comparison of the number of electropolymerization cycles.

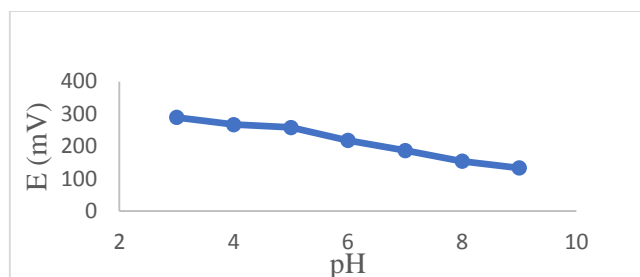
Number of Cycles	$I_p$ ( $\mu\text{A}$ )	E(V)
5	29.08	0.9800
10	40.57	0.9820
15	47.42	0.9920



**Fig. 2.** Cyclic voltammogram of the number of electropolymerization cycles.

### 3.4 Determination of The Optimum pH

The optimization of pH is done by measuring the potential of rhodamine B solution with a concentration of  $10^{-4}$  M with a pH in the range of 3-9. At low pH, rhodamine B is in the form of molecules and glutamic acid monomers are in a protonated form so that the active site of the rhodamine B mold contained in the MIP polymer matrix will be more selective in recognizing the structure of the rhodamine B molecule in the form of cations. Whereas in alkaline conditions, the reaction is initiated by the transfer of anomeric protons to bases [21]. The resulting pH is shown in **Figure 3**.



**Fig. 3.** The pH optimization curve for measurement of  $10^{-4}$  M rhodamine B solution using EPK-MIP potentiometrically.

The pH optimization curve shows a potential decrease with an increase in pH. But at pH 4-5 the electrode potential is relatively constant, so this pH 4-5 is the optimum pH measurement.

## 4 Conclusions

MIP modified carbon paste electrodes with rhodamine B as template molecules can be made using the cyclic voltammetry method to analyze rhodamine B in a potentiometric manner. The optimum number of cycle in electropolymerization is 15 cycles with 3 mM glutamic acid: 1 mM Rhodamine B in phosphate buffer pH 4.

**Acknowledgments.** The authors are very grateful to Hibah PDUPT 2019 Ristekdikti for their financial support for this research study.

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